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- (21) International Application Number: PCT/GB03/00253 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
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- (75) Inventors/Applicants (*for US only*): **ROSS, Richard** [GB/GB]; Chemical Sciences, Northern General Hospital, Sheffield S5 7ALL (GB). **ARTYMIUK, Peter** [GB/GB]; University of Sheffield, Dept. of Molecular Biology and Biotechnology, Sheffield S10 2TN (GB). **SAYERS, Jon** [GB/GB]; University of Sheffield, Division of Genomic Medicine Infection & Immunity, Medical School, F Floor, Beech Hill Road, Sheffield S10 2RX (GB).
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(54) Title: MULTIMERS OF RECEPTOR-BINDING LIGANDS

(57) Abstract: The invention relates to the provision of oligomeric polypeptides (dimers, trimers, etc) comprising the ligand binding domains of cytokines which are linked via flexible polypeptide linker molecules. The linker molecules optionally comprise protease sensitive sites to modulate the release of biologically active cytokines when administered to a human or animal subject.

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In national Application No

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/52 C12N15/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, CHEM ABS Data, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 23615 A (BUNDESREP DEUTSCHLAND ;BAIER MICHAEL (DE); LANG KURT (DE); METZNER) 3 July 1997 (1997-07-03)	20
Y	the whole document	1,2, 9-19, 21-23, 26-29, 39-42
Y	WEICH N S ET AL: "INTERLEUKIN-3/ERYTHROPOIETIN FUSION PROTEINS: IN VITRO EFFECTS ON HEMATOPOIETIC CELLS" EXPERIMENTAL HEMATOLOGY, NEW YORK, NY, US, vol. 21, no. 5, May 1993 (1993-05), pages 647-655, XP000983864 ISSN: 0301-472X page 650, left-hand column	1,2,5,8, 9,11-15, 18-24, 29,32-42

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>LIESCHKE G ET AL: "Bioactive murine and human interleukin-12 fusion proteins which retain antitumor activity in vivo" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 15, no. 1, January 1997 (1997-01), pages 35-40, XP002106574 ISSN: 1087-0156 the whole document</p>	1,2,5,8, 9,11-15, 18-24, 29,32-42
Y	<p>WO 00 37642 A (DAVIS SAMUEL J ;GALE NICHOLAS W (US); STAHL NEIL (US); REGENERON P) 29 June 2000 (2000-06-29)</p> <p>page 26 -page 27; claims</p>	1,2,5,8, 9,11-15, 18-24, 29,32-42
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Y	<p>US 5 525 491 A (OPPERMANN HERMANN ET AL) 11 June 1996 (1996-06-11)</p> <p>the whole document</p>	1,2,5,8, 9,11-15, 18-24, 29,32-42
Y	<p>WO 92 03569 A (SANGSTAT MEDICAL CORP) 5 March 1992 (1992-03-05)</p> <p>page 4 -page 12; claim 9</p>	1,2,5,8, 9,11-15, 18-24, 29,32-42
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Y	<p>US 6 037 329 A (CHANDLER LOIS ANN ET AL) 14 March 2000 (2000-03-14)</p> <p>column 32, line 62 -column 33, line 20 column 42, line 54 -column 43, line 44; claim 20</p>	1,2,5, 8-15, 18-24, 29,32-42

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INTERNATIONAL SEARCH REPORT

Ir. International Application No

PCT/83 03/00253

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>GUAN K ET AL: "EUKARYOTIC PROTEINS EXPRESSED IN ESCHERICHIA-COLI AN IMPROVED THROMBIN CLEAVAGE AND PURIFICATION PROCEDURE OF FUSION PROTEINS WITH GLUTATHIONE S-TRANSFERASE" ANALYTICAL BIOCHEMISTRY, vol. 192, no. 2, 1991, pages 262-267, XP009013567 ISSN: 0003-2697</p> <p>-----</p>	

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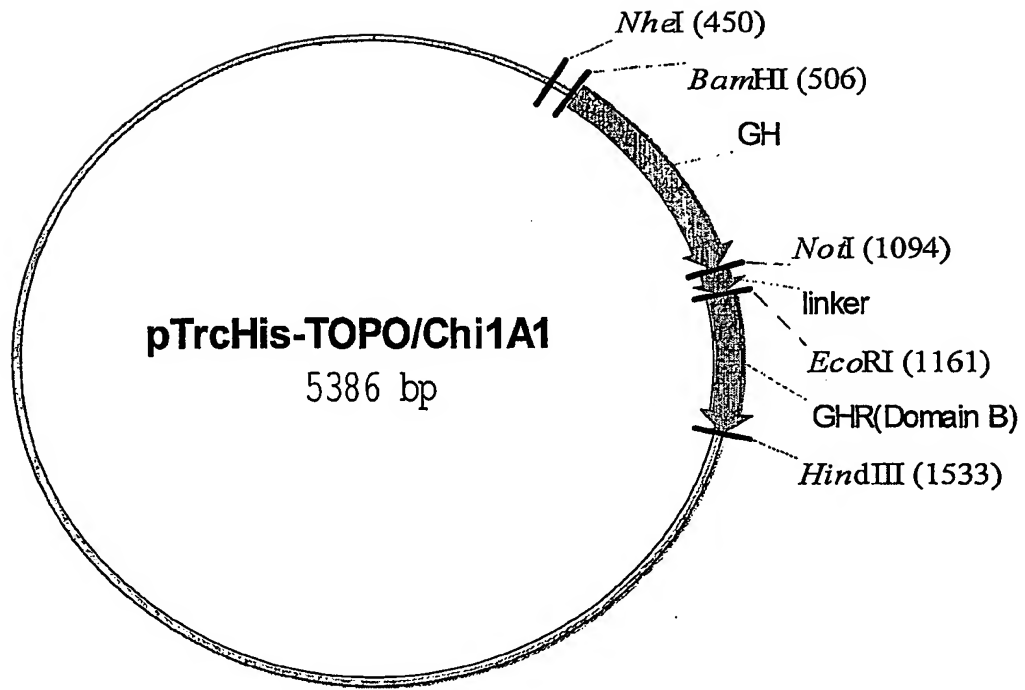


FIGURE 1

Name	5'-Sequence-3'
DiGHEcoF	AGGCGAATTCTTCCCAACCATTCCCTAT (SEQ ID:7)
DiGHNotF	CTTCAAGAGGCGGCGGCCGCTTCCCAACCATTCCCTTAT (SEQ ID:8)
DiGHHinR	TTCCAAGCTTCATCAGAAGCCACAGCTGCCCTCCA (SEQ ID:9)
Lep2TrcFOR	CAAAGCTAGCGGTGGCATGCAAGT (SEQ ID:10)
Lep2TrcREV	AAGCTTGAATTCCTATTACGTCGACTCTAG (SEQ ID:11)
LepLinkFOR	CAGCTGCTGTGGCTTCGGCGGCCGCAGGTGGCGGA (SEQ ID:12)
LepLinkREV	AATGCCTCGAGGAATTCGGAACCTCCG (SEQ ID:13)
Lep2FOR	GGGAAACTCGAGGTGCCCATCCAAAAAGTCCAAGAT (SEQ ID:14)
Lep2REV	GGGAAAGTCGACTCTCTAGAGCACCCAGGGCTGAGGTCC (SEQ ID:15)

FIGURE 2

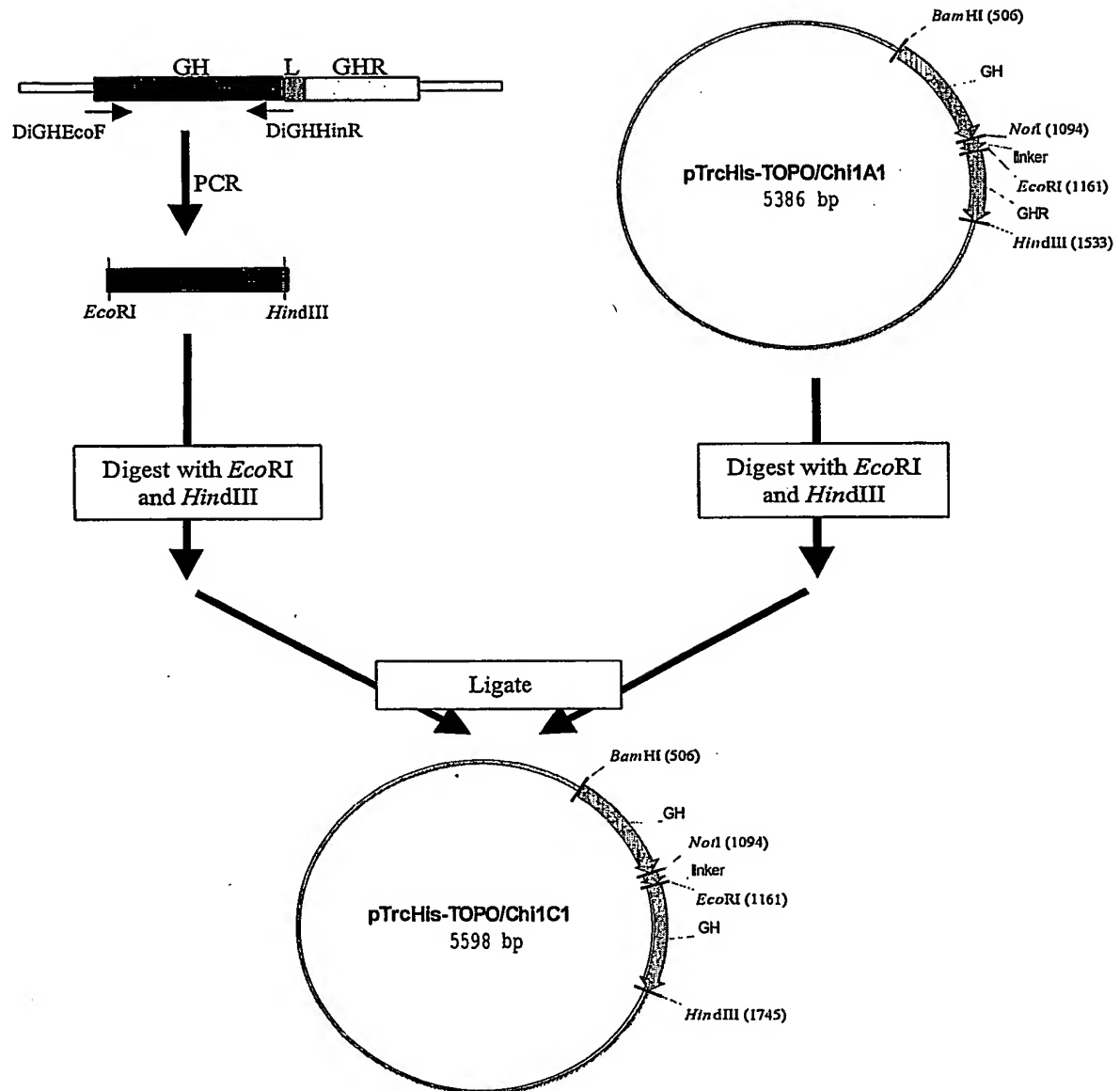


FIGURE 3

Growth Hormone Molecule 1

TTCCCAACCATTTCCCTTATCCAGGCTTTTTGACAACGCTAGTCTCCGCGC
CCATCGTCTGCACCAGCTGGCCTTTGACACCTACCAGGAGTTTGAAGAAG
CCTATATCCCAAAGGAACAGAAGTATTCATTCTGCAGAACCCCCAGACC
TCCCTCTGTTTCTCAGAGTCTATTCCGACACCCTCCAACAGGGAGGAAAC
ACAACAGAAATCCAACCTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCC
AGTCGTGGCTGGAGCCCGTGACAGTTCCCTCAGGAGTGTCTTCGCCAACAGC
CTGGTGTACGGCGCCTCTGACAGCAACGTCTATGACCTCCTAAAGGACCT
AGAGGAAGGCATCCAAACGCTGATGGGGAGGCTGGAAGATGGCAGCCCCC
GGACTGGGCAGATCTTCAAGCAGACCTACAGCAAGTTCGACACAACTCA
CACAACGATGACGCACTACTCAAGAACTACGGGCTGCTCTACTGCTTCAG
GAAGGACATGGACAAGGTCGAGACATTCCTGCGCATCGTGCAGTGCCGCT
CTGTGGAGGGCAGCTGTGGCTTC (SEQ ID:16)

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TTAGTGCCGCGCGGCAGTCCGGGCATTGGCGGCGGTGGCGGC (SEQ ID:17)

Growth Hormone Molecule 1

TTCCCAACCATTTCCCTTATCCAGGCTTTTTGACAACGCTAGTCTCCGCGC
CCATCGTCTGCACCAGCTGGCCTTTGACACCTACCAGGAGTTTGAAGAAG
CCTATATCCCAAAGGAACAGAAGTATTCATTCTGCAGAACCCCCAGACC
TCCCTCTGTTTCTCAGAGTCTATTCCGACACCCTCCAACAGGGAGGAAAC
ACAACAGAAATCCAACCTAGAGCTGCTCCGCATCTCCCTGCTGCTCATCC
AGTCGTGGCTGGAGCCCGTGACAGTTCCCTCAGGAGTGTCTTCGCCAACAGC
CTGGTGTACGGCGCCTCTGACAGCAACGTCTATGACCTCCTAAAGGACCT
AGAGGAAGGCATCCAAACGCTGATGGGGAGGCTGGAAGATGGCAGCCCCC
GGACTGGGCAGATCTTCAAGCAGACCTACAGCAAGTTCGACACAACTCA
CACAACGATGACGCACTACTCAAGAACTACGGGCTGCTCTACTGCTTCAG
GAAGGACATGGACAAGGTCGAGACATTCCTGCGCATCGTGCAGTGCCGCT
CTGTGGAGGGCAGCTGTGGCTTC (SEQ ID:16)

FIGURE 4

Growth hormone molecule 1

FPTIPLSRLFDNASLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQT
SLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANS
LVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNS
HNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF (SEQ ID:18)

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LVPRGSPGIGGGGG (SEQ ID:19)

Growth hormone molecule 1

FPTIPLSRLFDNASLRAHRLHQLAFDTYQEFEEAYIPKEQKYSFLQNPQT
SLCFSESIPTPSNREETQQKSNLELLRISLLLIQSWLEPVQFLRSVFANS
LVYGASDSNVYDLLKDLEEGIQTLMGRLEDGSPRTGQIFKQTYSKFDTNS
HNDDALLKNYGLLYCFRKDMDKVETFLRIVQCRSVEGSCGF (SEQ ID:18)

FIGURE 5

Leptin molecule 1

GTGCCCATCCAAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACAAT
TGTCACCAGGATCAATGACATTTACACACGCAGTCAGTCTCCTCCAAAC
AGAAAGTCACCGGTTTGGACTTCATTCTGGGCTCCACCCCATCCTGACC
TTATCCAAGATGGACCAGACACTGGCAGTCTACCAACAGATCCTCACCAG
TATGCCTTCCAGAAACGTGATCCAAATATCCAACGACCTGGAGAACCTCC
GGGATCTTCTTCACGTGCTGGCCTTCTCTAAGAGCTGCCACTTGCCCTGG
GCCAGTGGCCTGGAGACCTTGGACAGCCTGGGGGGTGTCTTGGAAGCTTC
AGGCTACTCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGTCTCTGC
AGGACATGCTGTGGCAGCTGGACCTCAGCCCTGGGTGC (SEQ ID:20)

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TTAGTGCCGCGCGGCAGTCCGGGCATTGGCGGCGGTGGCGGC (SEQ ID:17)

Leptin molecule 2

GTGCCCATCCAAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACAAT
TGTCACCAGGATCAATGACATTTACACACGCAGTCAGTCTCCTCCAAAC
AGAAAGTCACCGGTTTGGACTTCATTCTGGGCTCCACCCCATCCTGACC
TTATCCAAGATGGACCAGACACTGGCAGTCTACCAACAGATCCTCACCAG
TATGCCTTCCAGAAACGTGATCCAAATATCCAACGACCTGGAGAACCTCC
GGGATCTTCTTCACGTGCTGGCCTTCTCTAAGAGCTGCCACTTGCCCTGG
GCCAGTGGCCTGGAGACCTTGGACAGCCTGGGGGGTGTCTTGGAAGCTTC
AGGCTACTCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGTCTCTGC
AGGACATGCTGTGGCAGCTGGACCTCAGCCCTGGGTGCTTAGTGCCG (SEQ ID:21)

FIGURE 6

Leptin molecule 1

VPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVTGLDFIPGLHPILT
LSKMDQTLAVYQQILTSMPSRNVIQISNDLENLRDLLHVLAFSKSCHL
PWASGLETLDLGGVLEASGYSTEVVALSRLQGSLQDMLWQLDLSPGC (SEQ ID:22)

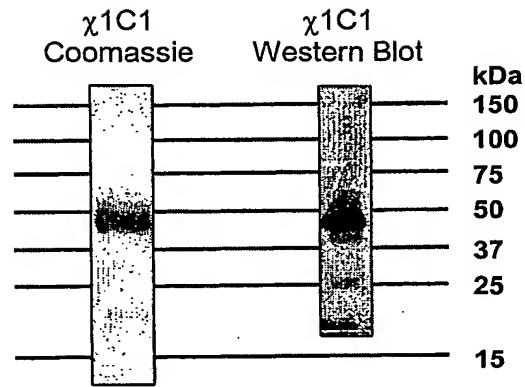
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LVPRGSPGIGGGGG (SEQ ID:19)

Leptin molecule 2

VPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVTGLDFIPGLHPILT
LSKMDQTLAVYQQILTSMPSRNVIQISNDLENLRDLLHVLAFSKSCHL
PWASGLETLDLGGVLEASGYSTEVVALSRLQGSLQDMLWQLDLSPGC (SEQ ID:22)

FIGURE 7

**FIGURE 8**

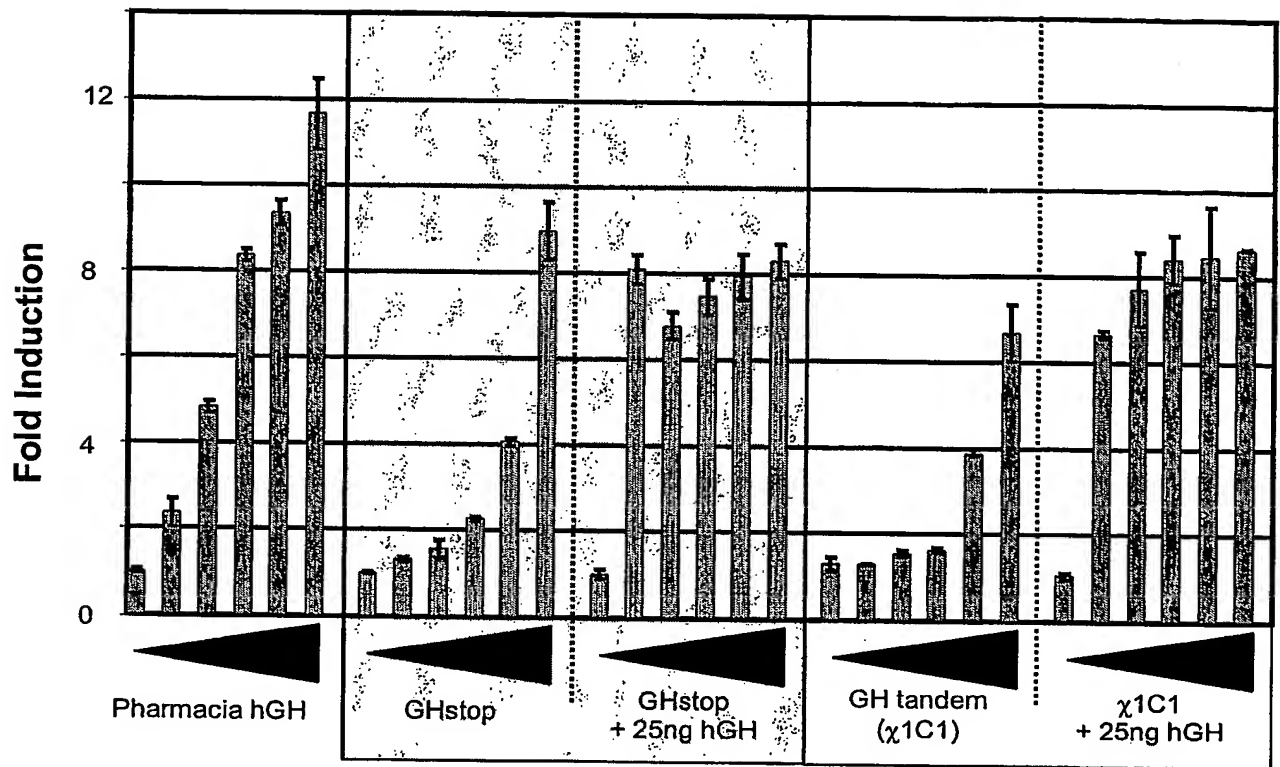


FIGURE 9

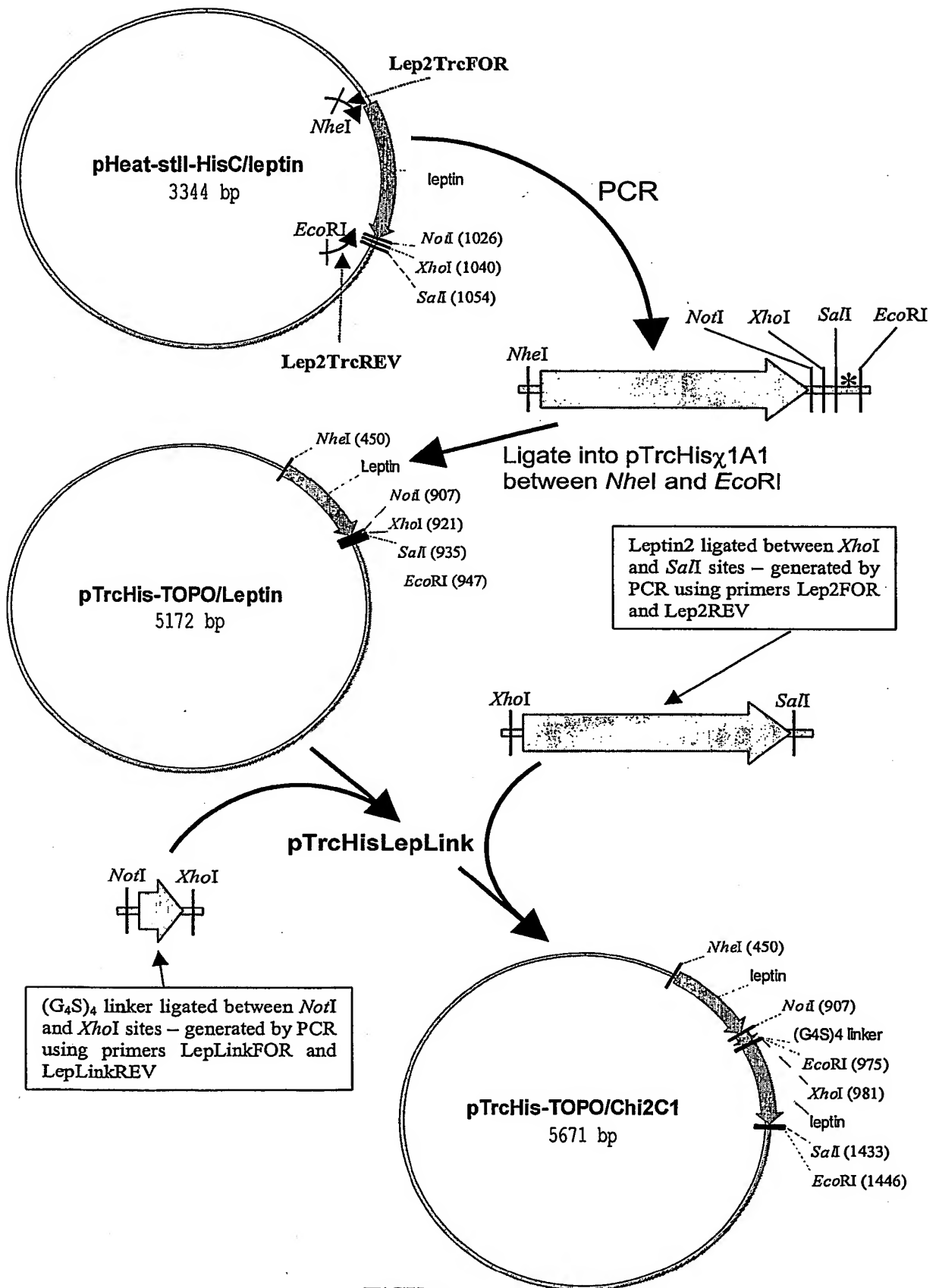


FIGURE 10

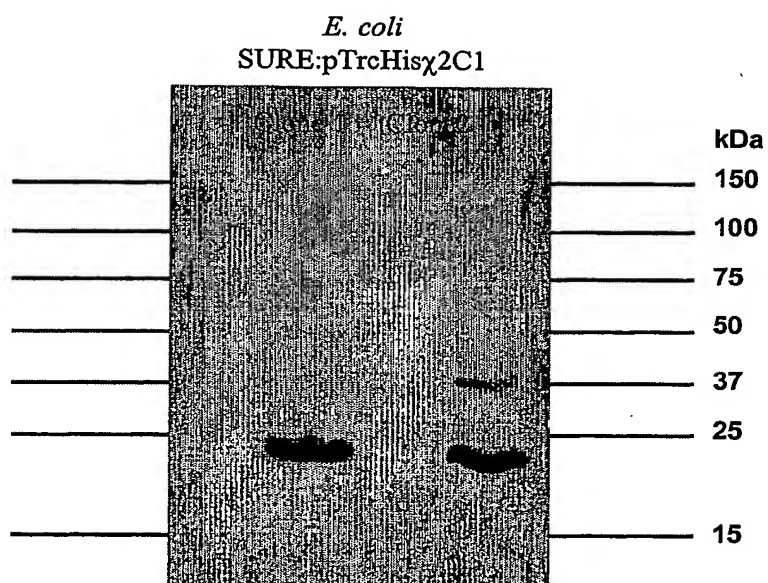


FIGURE 11

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